AGAROSE AND AGAROPECTIN IN GELIDIUM-AND GRACILARIA-AGAR

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By

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Since gel strength is the most important property of agar, *Gelidium amansii* is in great demand as a valuable raw material to meet this requirement in the agar industry of Japan and Korea. The other widely used red seaweeds, ten or more in species, have been employed only as admixtures with the above basal weed. There appeared, however, a new method for utilizing some subsidiary weed, such as *Gracilaria verrucosa*, as the second chief raw material to yield a high setting power of gel in Japan after World War II. It depends on treating the weed concerned with an alkaline solution prior to the usual processing.

In 1929 Haas and Russel-Wells (1) have found that the mucilaginous substance from certain weeds became high in gelling ability when subjected to alcoholic potash action. Yanagawa (1938) (2) has studied the effect of sodium hydroxide on the mucilages and found that among thirty species of weeds tested *Gracilaria verrucosa*, *G. gigas* and *Pterocladia tenuis* were considerably high in gel strength. He thought that this alkali treatment probably caused the conversion of the algal polysaccharide from low setting power of gel to high which is similar to that obtained with *Gelidium amansii* after splitting off a large proportion of sulphuric acid from its sulphate ester. Despite his indications, an increase in the quantity of released sulphate was not always parallel to the rise in gel strength. In fact, the sulphate ester of *Gracilaria gigas* and *Pterocladia tenuis* was stable toward alkali in his experiments.

It is very interesting to note that the alkali treatment promises to become a valid method for utilizing certain kinds of weeds as the chief raw material. It is true of *Gracilaria verrucosa* as mentioned above in Japan and Korea. In 1951 Kojima and Funaki (3) reported that the addition of calcium chloride to an alkaline solution was more effective in promoting gelling ability.

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The primary structure of agar has been determined by Araki's extensive studies (4). He has shown that agar consists of two fractions, a neutral polymer (agarose) and a sulphated polysaccharide (agaropectin). The gelling ability was found to be principally attributable to the former. Thus the *Gelidium amansii*- and alkali-treated *Gracilaria sp.*-agar have been studied on the properties of agarose and agaropectin with reference to the setting power of gel.

1. Ratio of agarose to agaropectin

Materials used were the agars made from Korean *Gelidium amansii* and alkali-treated Chilian *Gracilaria sp.* by the processing method as usual. KG and ATCG are used as the abbreviations for the former and latter agar respectively in the following. Alkali treatment was carried out as follows: cleaned weed is treated with one per cent solution of sodium hydroxide at 90°C for about 2 hours, neutralized with a dilute solution of hydrochloric acid and then washed with water.

Agarose and agaropectin were determined by Araki's acetylation method.

The results are summarized in Table 1. It shows that the ratio of acetylated agarose to acetylated agaropectin was about 1.5: 1 in KG and about 20: 1 in ATCG. It means ATCG contained a larger amount of agarose and a smaller amount of agaropectin than in KG.

Table 1. The ratio of agarose to agaropectin in agar.

Expt. No.	Materials	Acetylated agarose (%)	Acetylated agaropectin (%)	Acetylated agarose / Acetylated agaropectin
1	*Gelidium* agar	50.0	39.5	1.3
2	〃	56.7	34.4	1.6
3	*Gracilaria* agar	75.7	3.6	21.0
4	〃	75.5	3.8	19.9

2. Infrared spectra of agar, agarose and agaropectin

Infrared absorption spectra were measured on KG, ATCG, the mucilage from intact Chilian *Gracilaria*, agaroses from KG and ATCG and agaropectin from KG by the film method using Jasco model IR-S infrared spectrophotometer. As seen in Figures 1 and 2, the infrared spectra of both agars and agaroses and of agaropectin are almost similar to each other, but the mucilage extracted from non-alkali-treated *Gracilaria* displayed a characteristic absorption at 1540cm^{-1} indicating an amide deformation. Disappearance of this band in ATCG and KG may be due to the removal of some peptide from the algal polysaccharide by the action of alkali or in the course of preparing agar.

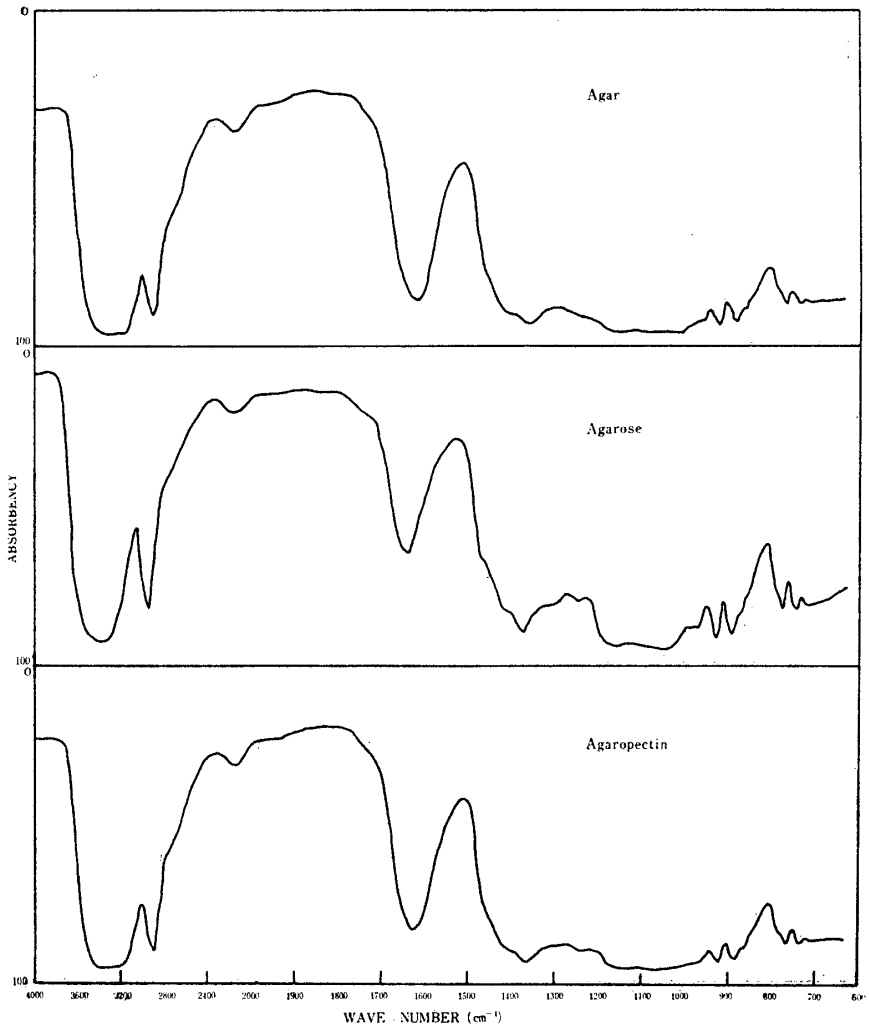

Fig. 1 Infrared absorption spectra of *Gelidium amansii*-agar, -agarose and -agaropectin.

3. Specific rotation of acetylated agarose

Acetylated agaroses from KG and ATCG had a specific rotation of –30° and –29° at 15°C respectively.

4. Gel strength of agar, agarose and agaropectin

Gelling ability was measured on 1.5% gel by Nikkansui Shiki method using Nikkansui Shiki gelometer.

The ATCG was greater in gel strength than the KG. Both agarose and agaropectin separated from agar had the ability to form gel, but reduced the gel strength and visco-elasticity by comparison with the starting agar. This may be mainly due to the depolymerization occurred in the isolation process, especially acetylation step at relatively high temperature. The same can be said of the

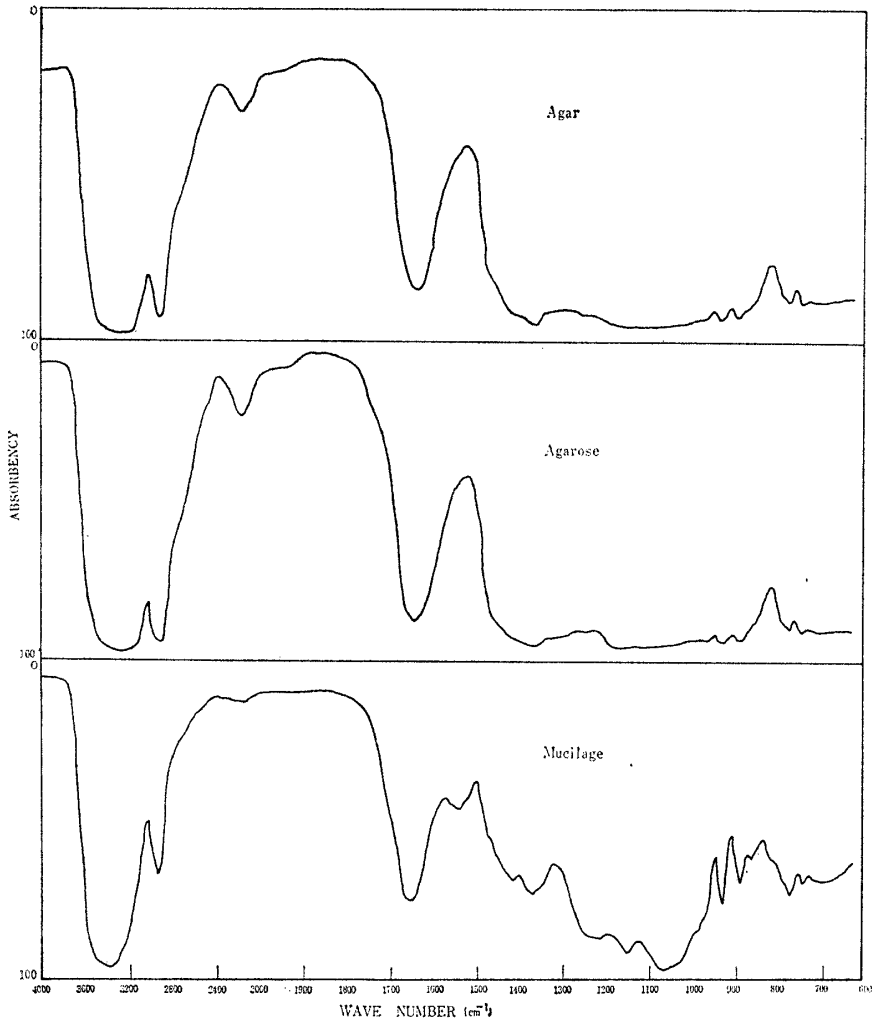

Fig. 2 Infrared absorption spectra of alkali-treated *Gracilaria*-agar and -agarose and mucilage extracted from intact Gracilaria sp.

agarose prepared by the method of Hjerten using cetylpyridinium chloride (5). On the contrary, the agarose fractioned by the Russel's polyethylene glycol method (6) was considerably high in gel strength while the agaropectin was very low as indicated in Table 2.

5. Molar ratio of galactose to 3,6-anhydrogalactose in agar, agarose and agaropectin.

The contents of sugars in algal polysaccharide were determined by Yaphe's method (7). The results obtained are summarized in Table 2. It shows that the molar ratio of galactose to 3,6-anhydrogalactose in KG, ATCG, agaroses from KG and ATCG and agaropectin obtained from KG had the value of about 1.0–1.2: 1. However, the ratio was less than 1 in the agarose fractioned by Russel's polyethylene glycol method and its gel strength became twice high that of the starting agar,

Table 2. Molar ratio of galactose to 3,6-anhydrogalactose in agariferous materials.

Materials	Galactose (%)	3.6-anhydro-galactose (%)	Molar ratio of galactose to 3,6-anhydro-galactose	Gel strength (g/cm^2)
Gelidium agar	39.4	35.5	1.10	550
〃 agarose (1)	55.4	45.7	1.21	420
〃 〃 (2)	40.0	48.2	0.83	1100
〃 〃 (3)	44.6	35.8	1.25	low
〃 agaropectin (1)	53.1	41.1	1.26	390
〃 〃 (2)	60.6	12.3	4.92	low
Alkali-treated *Gracilaria* agar	39.4	46.5	0.85	910
〃 agarose (1)	56.0	46.9	1.19	540
Non-alkali-treated *Gracilaria* agar	66.2	23.7	2.79	low
Non-alkali-treated Argentine *Gracilaria* agar	48.0	36.8	1.30	300
Ceramium agar	50.0	47.9	1.04	550
Hypnea mucilage	50.9	25.6	2.00	low

Note.
(1): prepared by the acetylation method of Araki.
(2): prepared by the polyethylene glycol method of Russel *et al.*
(3): prepared by the cetylpyridinium chloride method of Hjerten.

while the agaropectin was considerably low and the ratio of galactose to 3,6-anhydrogalactose had the value of 4.92. The mucilages from *Hypnea charoides* and non-alkali-treated *Gracilaria verrucosa*, which were extremely low in gel strength, were comparatively high being 2.0 and 2.8 respectively.

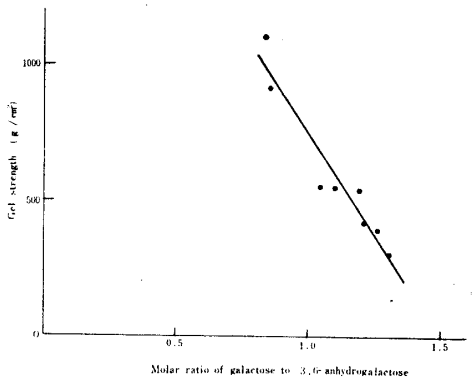

Fig. 3 A relationship between the molar ratio of galactose to 3,6-anhydrogalactose and gel strength.

There was found a linear rise in the value of molar ratio accompanied by a decrease in gel strength as seen in Figure 3. Thus the measurement of this

value is recommended as a useful method for determining the quality of agar with special reference to the gel strength.

The reason that ATCG is higher in gel strength than the intact Chilian *Gracilaria* agar is considered to be mainly due to the fact that the algal polysaccharide concerned became rich in agarose content resulting from the removal of agaropectin during the alkali treatment or the following process of making agar. The report dealing with this subject shall be presented in detail elsewhere.

Summary

1. The ratio of agarose to agaropectin differed greatly according to the varieties of agar.

2. The infrared spectra of agar, agarose and agaropectin from *Gelidium amansii* and *Gracilaria sp.* were similar.

3. The gel strength was principally attributable to that of agarose in the agar concerned. Among three methods using acetic anhydride, cetylpyridinium chloride or polyethylene glycol for fractionation of agarose and agaropectin, the last one was the best in obtaining a high setting power of agarose gel.

4. There was found a linear rise in the value of molar ratio of galactose to 3,6-anhydrogalactose in agariferous materials accompanied by a decrease in gel strength.

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